Application No. 10/518,723
Attorney Docket No 2226-045890

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/518,723	Confirmation No. 1009
Applicant	:	NICOLAAS DUNEAS	
Filed	:	August 10, 2005	
Title	:	METHOD OF PREPARING AN OSTEOGENIC PROTEIN FRACTION	
Group Art Unit	:	1646	
Examiner	:	Elizabeth C. Kemmerer	
Customer No.	:	28289	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
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Alexandria, VA 22313-1450

I, Nicolaas Duneas, declare as follows:

1. I am a citizen of the Republic of South Africa (Country) and reside at 204 Groenkloof Gardens Pretoria (Address). I graduated from Boksburg High School (School) and received a Doctor of Philosophy (Degree) in 1998 (year). I have 10 years of experience within the field of tissue engineering, working for Altis Biologics (company).

2. I have read and am thoroughly familiar with the contents of the above-identified patent application. Furthermore, I have read and understand the Office Actions dated April 28, 2008 and October 30, 2008 and the issues and prior art references listed therein, specifically Scott et al. (1994, The Anatomical Record 238:23-30) (hereinafter "the Scott reference") or Yoshimura et al. (1993, Biol. Pharm. Bull. 16(5):444-447) (hereinafter "the Yoshimura reference") in view of United States Patent No. 4;968,590 to Kuberasampath et al. (hereinafter "the Kuberasampath patent").

3. Contrary to the assertions in the Office Actions dated April 28, 2008 and October 30, 2008, utilization of a 300 kDa yields unexpected results thereby making the claimed invention non-obvious in view of Scott or Yoshimura in view of Kuberasampath.

4. Claim 36 is directed to a method of preparing an osteogenic protein fraction, extracting demineralized bone matrix with a solution of at least one chaotropic agent; removing high molecular weight proteins which exceed 300 kDA from the extract by ultrafiltration with a 300 kDA membrane to produce a lower molecular weight fraction; subjecting the lower molecular weight fraction to heparin affinity chromatography under conditions which first favor the binding and then the elution of a purified heparin affinity fraction containing the osteogenic protein fraction; subjecting the heparin affinity fraction to hydroxyapatite chromatography under conditions which first favor the binding and then the elution of a purified osteogenic protein fraction; and exchanging the purified osteogenic protein fraction into a solvent suitable for human medical use. The chaotropic agent is selected from the group consisting of urea and guanidinium salts to produce an extract.

5. The evidence pointing to the high yield of BMPs produced by the method of the claimed invention is clearly apparent when considering the empirical data generated in pilot experiments. Figure 1 is a chromatogram of the heparin-affinity procedure using the 300 kDA membrane of the invention. The unbound fraction (large arrow) is typically larger than that of the 100 kDA membrane (not shown) since more total protein passes through the larger pores of the 300 kDA membrane. The unbound (large arrow) is likewise substantially reduced following removal of collagenous peptides, and the osteogenic fraction (small arrow) contains four times more protein than that produced in the method of Kuberasampath, and an estimated two-fold more protein when using a 100 kDA membrane.

6. The advantage gained using the method of the present invention can be clearly distinguished by the hydroxyapatite chromatography data shown in Figure 2. The chromatograms shown in Figure 2a and Figure 2b, which are for two different batches of BMPs, show the larger relative size of the BMP fraction (large arrow) compared to the size of the unbound fraction (smaller arrow). A direct comparison with the chromatographic profiles of Kuberasampath (Figure 2c) shows a much larger relative size of the unbound fraction,

indicating that the present invention leads to greatly enhanced recovery of osteogenic fraction. Using ELISA assay and antibodies for BMP-2 for four other experiments, the Applicant has found that a total final recovery of an average of 41% of the total BMP in the original raw material (Table 1) is recovered into the final product, confirming the high yield capabilities of the process. When applied to single donor bone technology (Figure 1), yields as high as 75% have been achieved.

Table 1.. Yield data of empirical experiments using 300 kDA membrane.

Lot #	estimated dry DBM kg	estimated total hBMP-2 @25 ng/g DBM (ng)	estimated natural abundance hBMP-2 @ 25ng/g DBM	total hBMP complex (mg)	total hBMP-2 purified ng	percent yield hBMP-2 from estimated total
Lot 01 - 2006	1.5	22500.0	0.00000250%	51	12236	54.4%
Lot 02 - 2006	3.0	45000.0	0.00000250%	62.608	11774	26.2%
Lot 03 - 2006	1.7	24750.0	0.00000250%	80.35	9935	40.1%
Lot 04 - 2006	1.5	22500.0	0.00000250%	73.695	10336	45.9%
average						41.7%

Rapid processing is also important for the collection of a high activity of BMP.

7. The use of a 100 kDA membrane, as in Scott and Yoshimura, requires:

(a) higher transmembrane pressures;

(b) higher throughput volume of the entire system, i.e., higher number of passes of the extract over the membrane;

(c) a longer period of time to complete the ultrafiltration step; and

(d) interventions to minimize fouling of the column by collagens and collagen aggregates, which would affect the flux through the membrane which reduces the biological activity of the final product because:

(1) higher pressures result in higher shear forces exerted on the

BMP protein, and therefore higher rates of denaturation can be expected;

(2) longer retention times in the system increase the contact time of BMP and extracted proteases, leading to increased proteolysis of BMP by extracted proteases; and

(3) smaller effective pore size and greater fouling reduce the passage of the 30 kDA BMP through the membrane for a given amount of extract passes.

All of these disadvantages are overcome by the use of a 300 kDA membrane.

8. Because ultrafiltration with a 300 kDA membrane is not the same as filtering proteins with a 100 kDA membrane, as described by the Scott and Yoshimura references, the claimed invention achieves unexpected results and is not taught or suggested by the prior art.

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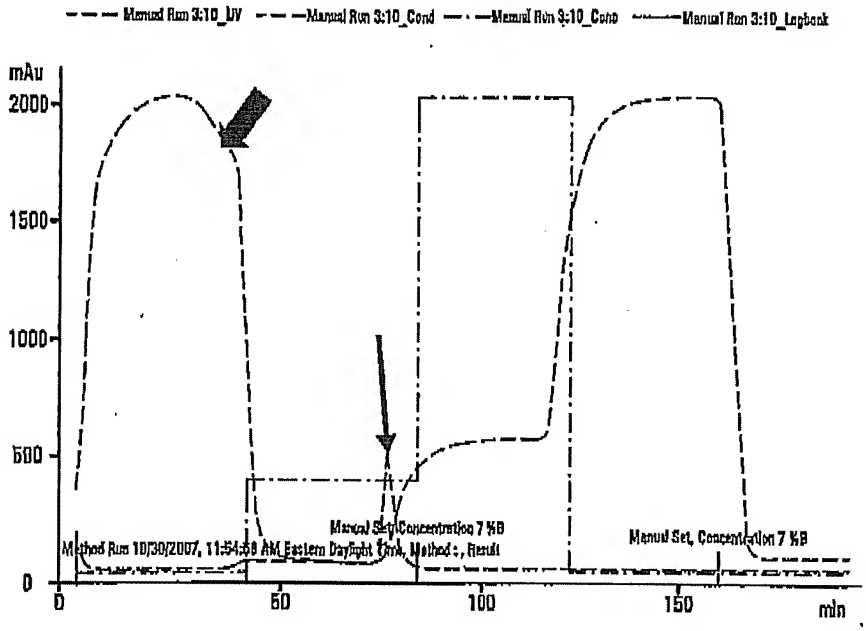

FIG 1

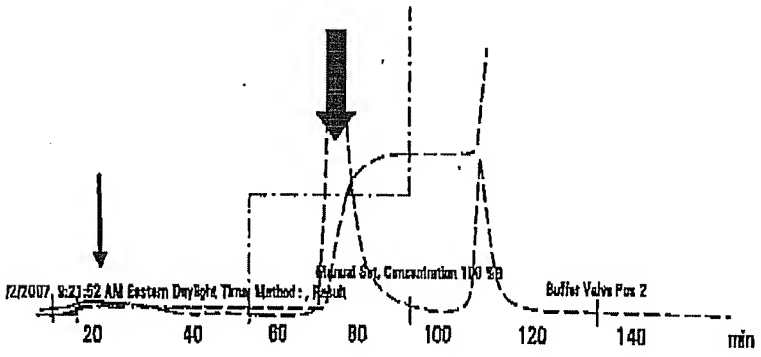

FIG 2a

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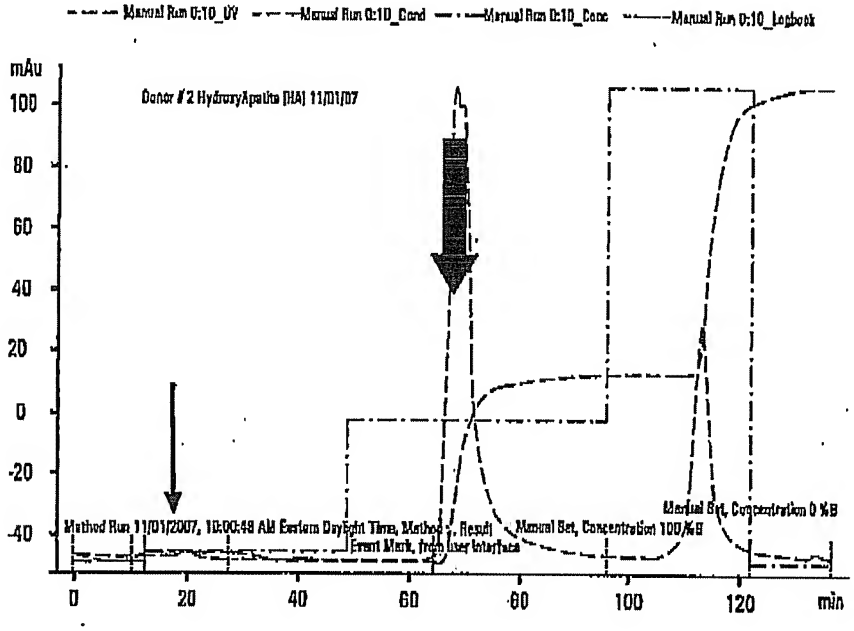

FIG 2b

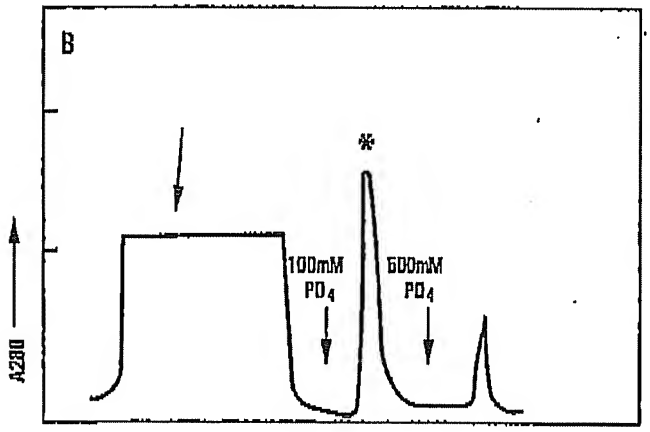

FIG 2c

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9. I declare further that all statements made herein of my own knowledge are true and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Respectfully submitted,

Nicolaas Duneas

28.01.2009

Date